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NITROGEN METABOLISM AND HUMAN NUTRITION.

BY

MANAYATH DAMODARAN,

(Biochemical Laboratory, University of Madras).

Part IV.

NITROGEN METABOLISM IN PLANTS.

Investigations on the nitrogen metabolism of plants have centred round asparagine, a substance first isolated in 1806 by Vauquelin and Robiquet from the juice of asparagus shoots. The reason for this interest was in the first instance due mainly to the accidental circumstance that asparagine is readily crystallised from plant extracts and can be detected microscopically in sections allowed to dry slowly. Its recognition as a ubiquitous plant constituent and the realisation of its importance in protein metabolism we owe to the pioneer researches of Piria (1844), Bossingault (1868) and Pfeffer (1872-1877) and the monumental work of Schulze (1875-1910).

Amino-acid changes in Seedlings.

A large part of the work of Schulze was carried out on the regeneration of protein in germinating seeds, i.e. on the processes by which during seedling growth the reserve protein of the seed was rebuilt into the protoplasmic protein of the seedling. The most important feature of this conversion was found to be the formation of large quantities of amides, in particular asparagine. The accumulation of asparagine was specially marked in etiolated seedlings kept in the dark by which means the new formation of protein was slowed down. Aspartic acid and asparagine are present in the protein molecule but in these experiments the amount of asparagine was far in excess of what could have been formed from the reserve proteins undergoing degradation. Thus in one experiment with 18-day-old etiolated lupin seedlings 50% of the nitrogen of the seedling was present in the form of asparagine. Analysis of seedlings at different ages showed that while protein and amino-acids decreased asparagine progressively increased.

From the fact that asparagine was found in larger quantities in etiolated than in normal seedlings and that in both the quantity decreased during active photosynthesis it was inferred that protein is synthesised from asparagine and carbon compounds derived from carbohydrates. The distribution of asparagine between the different parts of the plant pointed to the same conclusion. The cotyledons which are the seat of the hydrolysing reserves of proteins contained much amino-acid along with more asparagine than amino-acids showing that nitrogen was translocated from the cotyledons to the growing points in the form of asparagine to be there used for re-synthesis of protein. Similarly stems and petioles of plants contained more asparagine than leaves where it would be expected to be rapidly used up in synthesis of protein. In some seedlings, e.g. Ricinus, Cucurbita and many Cruciferae glutamine was found to take the place of asparagine.

Progress in protein chemistry which was taking place at the time of Schulze's work was making it increasingly clear that the units of the protein molecule are the amino-acids which are obtained free on acid hydrolysis.

Schulze did not envisage a different kind of hydrolysis to take place in plants, but postulated that amino-acids were the primary products of protein degradation in the seedling also and that these were later converted into asparagine. The fact that the amino-acids that could be isolated from the seedlings bore no relation to the amino-acids that were present in the original seed proteins indicated that they were converted at different rates into asparagine. There were other indications of the secondary origin of asparagine. Palladin (1888) and Susuki (1902) had both shown that oxygen is essential for asparagine synthesis. And Butkewitsch (1908) found that in seedlings anaesthetised with toluene instead of asparagine ammonia accumulates. Further Schulze himself was able to isolate many amino-acids from plants although only in small quantities.

Amino-acid changes in Ripening Seeds and Leaves.

Results analogous to those with seedlings on the importance of asparagine as a translocatory medium for protein synthesis were obtained with ripening seeds and with leaves. In the ripening seed there is a reversal of the processes taking place in germinations; soluble nitrogenous compounds pass from the leaves to the fruits and disappear there with protein formation. Emmerling who studied this process for a period of over twenty years showed that the seed pod acted as an intermediate storehouse of the nitrogenous substances which were later utilized in synthesis of seed protein. In the unripe seed there is relatively

little protein and a high proportion of soluble nitrogen, while in the ripe seed the reverse is the case.

Analysis by Schulze of the developing pods and seeds of *Pisum sativum* showed that during ripening there is a break-down of protein in the pod, the soluble nitrogenous compounds produced passing from the pod to the seed. Half the nitrogen of the pod was found to be asparagine. Other amino-acids—arginine, leucine, histidine and tryptophane—were also identified. In the seeds on the contrary all the amino-acids were identifiable, but little asparagine or glutamine, which were therefore assumed to have been utilized in the formation of protein.

As soluble nitrogenous compounds may pass directly from the leaves to the seed without previous storage in the pods amino-acid content of whole plants were also determined, when it was found that amino-acids were present in very low concentration while asparagine represented 40% of the non-protein nitrogen.

In leaves considerable accumulation of asparagine at the expense of protein was shown to take place in detached leaves and shoots kept for some days with their petioles dipping in water. There was first an increase of amino-acids which were then replaced by asparagine. Similar results were reported by Butkewitsch of *Avena sativa* and *Vicia faba* kept in the dark.

Schulze's view as finally summarised by him in 1906 could be put into modern terminology thus: Proteins are hydrolysed by the intracellular enzymes, the amino-acids produced are then oxidised with the production of ammonia which combines with some nitrogen-free residue derived either from carbohydrates or from amino-acids to form the amides of aspartic and glutamic acids. These are translocated to leaves and growing points where they are utilized for protein synthesis. Later investigations—the most important contributions are those of Priyanischnikov, Chibnall, Mothes, Vickery and their coworkers—have been concerned mainly with the confirmation of the experimental findings of Schulze and in throwing light on two important questions (1) The mechanism of asparagine formation and (2) The physiological significance of this substance. Neither of these problems has been satisfactorily solved.

The Mechanism of Asparagine Formation.

There is no doubt now that asparagine and glutamine pre-exist in the protein molecule. These substances have been isolated in *in vitro* experiments by digestion of proteins with suitable proteolytic enzymes. The presence of such enzymes in plant cells is also now well established so that the mechanisms for hydrolysing the reserve protein of seeds to amino-acids and asparagine and glutamine exist in the germinating seedling.

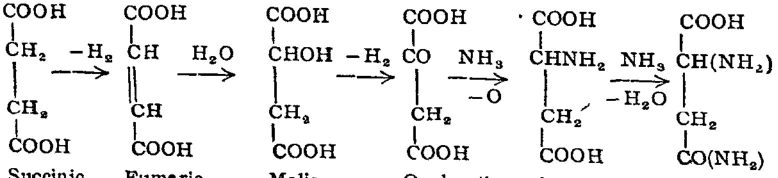
But it is also certain, at least in the case of asparagine, that the amount derivable by simple hydrolysis will not account for more than a fraction of the quantities usually observed.

(a) *Origin of the ammonia.*

From the facts (1) that asparagine accumulation in seedlings is accompanied by a fall in other amino-acids, (2) the necessity of oxygen for the process and (3) that ammonia accumulates when synthetic processes are inhibited by narcosis it has been concluded that the ammonia arises by an oxidative deamination of amino-acids analogous to that which takes place in the animal organism. But the mechanism of deamination still remains unknown. No enzyme corresponding to the amino-acid oxidases has been demonstrated in plants except in regard to one amino-acid, viz. glutamic acid. A glutamic acid dehydrogenase capable of superficially oxidising the amino-acid has been demonstrated in the three species of Leguminosae. A possible alternative agency for amino-acid oxidation has been suggested, viz. the quinones derived from phenols by the action of phenol oxidases which are widely distributed in the plant kingdom. But there is as yet little evidence to show that these do act in the manner suggested.

(b) *The Carbon skeleton.*

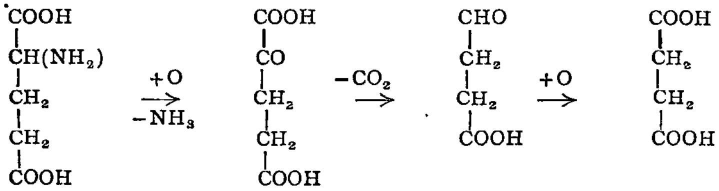
The four carbon dicarboxylic acids which are widely distributed in the plant kingdom, such as succinic and malic acids, have always been considered probable sources of the carbon required for asparagine synthesis. By analogy with the formation of amino-acids from keto-acids in animals the following scheme has been postulated:



Succinic Acid — Fumaric Acid — Malic Acid — Oxalacetic Acid — Aspartic Acid — Asparagine

There is evidence at present for the existence in plants of enzyme systems capable of catalysing all the above reactions. The succinic acid which is the starting point of this chain of reaction is usually assumed to arise from carbohydrate or fat metabolism. But there is also the possibility that in certain plants glutamic acid might be the precursor of succinic acid. The following reactions

by which glutamic acid could be converted into succinic acid have all been shown to be individually catalysed by plant enzymes :



Some support for this view is found in a recent study of the amino-acid changes in certain seeds during germination. It was found for example that in *Dolichos Biflorus* during seedling growth the total dicarboxylic amino-acid N (aspartic acid N— glutamic acid N) remains more or less constant although there is simultaneously a marked increase in asparagine suggesting a conversion of glutamic into aspartic acid.

Direct evidence of the participation of succinic acid or its derivatives in the synthesis of asparagine has been difficult to obtain. Using the vacuum infiltration technique of Bjorksten, Mothes was able to show that when ammonium succinate, fumarate or malate were infiltrated into the leaves of *P. multiferous* large amounts of asparagine were formed. Much of the value of this experiment was however abolished by later infiltration studies by Schwab in which it was shown that ammonium sulphate caused the same amount of amide formation as ammonium malate. Again infiltration experiments with ammonium aspartate and glutamate the same amount of asparagine was formed.

There is better evidence of a direct nature for the formation of glutamine from ketoglutaric acid. In 1933 Greenhill in Chibnall's laboratory found crystals of glutamine exuded on the leaf blades of rye grass grown in pot cultures which had received dressings of ammonium sulphate. Rye grass leaves were therefore considered a suitable material for studying the mechanism of glutamine synthesis. On infiltration with ammonium ketoglutarate excellent yields of glutamine were obtained; some asparagine also was formed. It was also shown that there was a corresponding disappearance of the keto-acid infiltrated.

Physiological Significance of Asparagine and Glutamine.

There are two opposing views on the physiological role of asparagine and glutamine in plants, one due to Schulze who considered that it was the chief translocatory medium for nitrogen and was more suited to protein synthesis than amino-acids, and the other originating with Priyanischnikov who considered that

asparagine served merely as a non-toxic form in which the ammonia required for protein synthesis could be stored prior to utilization. According to the former view asparagine is an obligatory intermediate product in the nitrogen metabolism of the plant; according to the latter it arises only under conditions in which ammonia is not immediately utilised for protein synthesis. The difference between Schulze and Priyanischnikov was not in regard to experimental results but with regard to their interpretation. As far as experimental findings were concerned Priyanischnikov confirmed the results of Schulze and in experiments of a different kind—water culture experiments in which barley seedlings were supplied with ammonium salts—demonstrated asparagine formation. Broadly speaking it may be said that in Schulze's conception the presence of a substance in large quantities in metabolising tissues indicated its usefulness in the chemical processes going on, while Priyanischnikov took the view that only substances that were not utilisable accumulated. The quantity of any substance which acts as an intermediate in metabolism will depend upon two factors, the speed with which it is formed and that with which it is utilised, so that by using balance-sheet methods of analysis it will be impossible to say whether asparagine is an obligatory intermediate product in protein formation or not.

Among the numerous recent investigations on the circumstances under which asparagine and protein synthesis take place and which might therefore throw light on the significance of asparagine formation the following may be mentioned:

(i) Chibnall has shown that there is a diurnal variation in the protein content of normal leaves, the protein increasing by day and decreasing by night, and further that there is a decrease of soluble nitrogen at night. These observations are interpreted thus:—Protein synthesis takes place actively in the leaves during the day when abundant carbohydrate is being photosynthesised. Simultaneously there is hydrolysis of protein and translocation of soluble substances from the leaf to the growing points. This is masked during the day by new synthesis of protein and becomes only apparent at night. The nature of the soluble products used for translocation is inferred from the products formed in detached leaves in the dark, i.e. they probably consist of the amides asparagine and glutamine.

(ii) Extensive researches were carried out by Mothes with special reference to the influence of carbohydrate on asparagine and protein synthesis. After confirming the known facts about the formation of amides in detached leaves kept in the dark, the necessity of oxygen for this process and its inhibition in narcosis, Mothes proceeded to show that in leaves rendered poor in

carbohydrate by prolonged darkening ammonia accumulated instead of amide and that when detached leaves which are not too old are exposed to daylight or fed with glucose in the dark amides disappeared and protein was formed. Leaves with abundant carbohydrate formed neither ammonia nor asparagine in narcosis. When leaves which were not too old were fed with ammonium sulphate from without, the fate of the ammonia depended upon the amount of carbohydrate present; with abundant carbohydrate the ammonia was rapidly synthesised to protein, with less carbohydrate it was stored as asparagine. In complete lack of carbohydrate the ammonia accumulated unaltered until the leaf died of ammonia poisoning. It was immaterial whether the carbohydrate was supplied by photosynthesis or by sugar feeding. When leaves were fed with asparagine it was converted into protein in the presence of abundant carbohydrate and in the absence of the latter was stored as such.

These experiments in the opinion of Mothes prove that asparagine has no special significance as a translocatory medium but is only a means of storing in innocuous form ammonia arising either from metabolism or supplied as a nutrient, which could not be immediately utilized for protein synthesis from lack of carbohydrate or other causes. They are generally considered to support Priyanischnikov's dictum that ammonia is the alpha and the omega in the nitrogen metabolism of plants. Ruhland and Wetzel from studies of a different nature came to the same conclusion. These authors consider that plants can be classified into acid plants and amide plants. In the former (example of which are Begonia and Rhubarb) the large amount of organic acids present enable the plant to stand high concentrations of ammonia so that no amide synthesis took place. This contention is however unlikely to be true as ammonium salts will give rise to ammonium ions which would still be poisonous. Further very careful researches of Vickery on the rhubarb plant have proved beyond doubt that in detached leaves synthesis of asparagine and glutamine take place as in other species previously studied.

The dicarboxylic acids in transamination and respiration.

It is obvious from the above discussion that the investigations so far carried out have not definitely demonstrated that the formation of the amides of dicarboxylic acids is an obligatory step in protein synthesis in the plant. There is however evidence of an indirect nature which makes it probable that the dicarboxylic acid and amino-acids play an important role in plant metabolism.

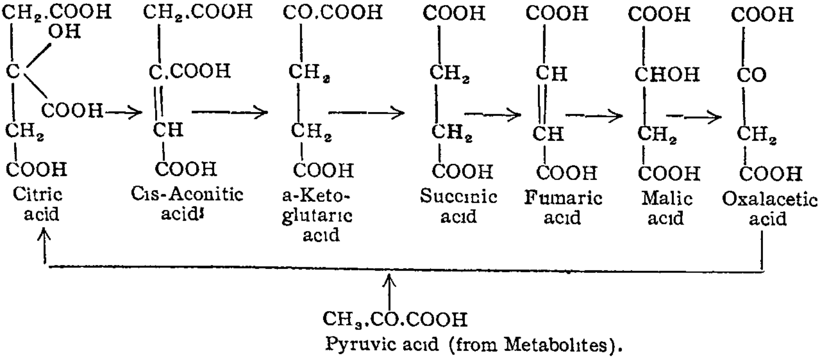
A biological reaction which brings about the transference of the amino groups of aspartic and glutamic acids to the keto-acids

corresponding to other amino-acids and vice versa has been described under the term transamination by the Russian workers, Braunstein and Kritzmann.

$$COOH-CH_2-CH(NH_2)-COOH + R-CO-COOH \rightleftharpoons COOH-CH_2-CO-COOH + R-CH(NH_2)COOH.$$

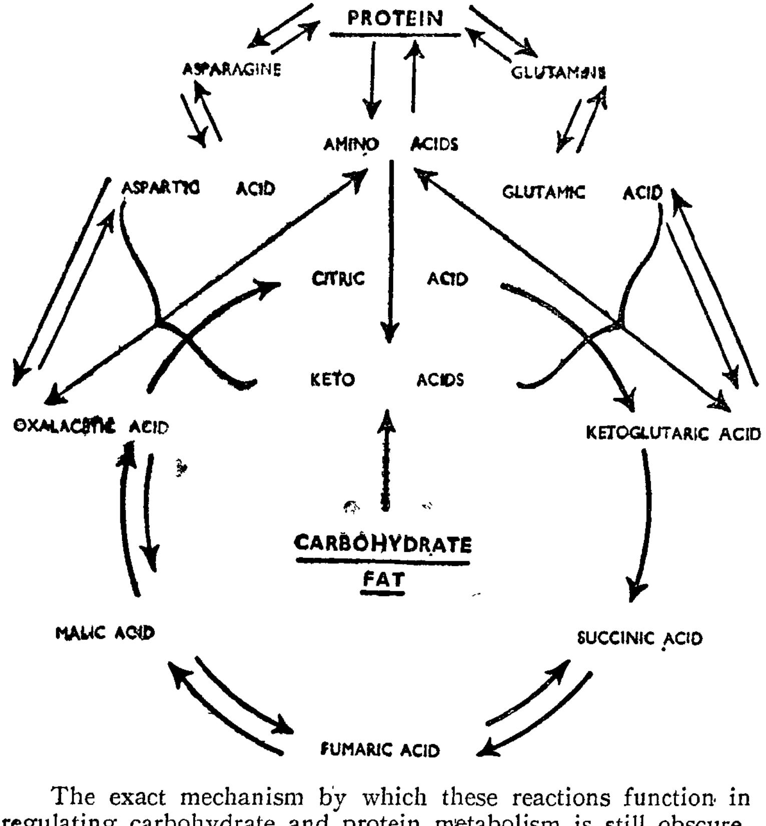
If, as has been claimed by these authors, the transaminase system is present in both plant and animal tissues then it is obvious that in metabolism all other amino-acids could arise from the dicarboxylic acids and further, under conditions in which large amounts of the former are formed they could be converted into the latter and accumulated in the form of asparagine and glutamine.

In respiration also the dicarboxylic acids have been shown to play a prominent part. The researches of Szent-Gyorgyi, Knoop and Krebs make it highly probable that in the normal oxidative processes taking place in respiration in animal cells the transfer of hydrogen from the metabolite to oxygen respired is effected through a cyclical series of reactions in which oxalacetic acid and ketoglutaric acids are essential intermediates. The scheme given below shows the citric acid cycle in respiration according to Krebs:



No experimental evidence has yet been produced to prove that the same respiratory cycle functions in the plant as in the animal cell. But the organic acids concerned especially citric, succinic, fumaric and malic acids are widely distributed in plants; the existence of the enzymes catalysing the oxidation of these substances has also been demonstrated in many species. It is therefore a probable hypothesis that the dicarboxylic amino-acids and the keto-acids derived from them oxalacetic acid and ketoglutaric acid serve as connecting links between protein and

carbohydrate metabolism in plants and animals. The inter-relations between these substances are indicated in the following diagram :



The exact mechanism by which these reactions function in regulating carbohydrate and protein metabolism is still obscure, but that they do so function appears extremely likely from the relationships between protein content and respiration which have been established by the researches of Gregory and others.

OSMOTIC REGULATION IN ANIMALS AND MAN

BY

N. KESAVA PANIKKAR *

Sir Subramania Ayyar Lectures, University of Madras 1943-44
Given in January 1944

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Part I

INTRODUCTION

Allow me first of all to express my deep appreciation of the honour this University have done me in inviting me to deliver the Sir Subramania Ayyar Lectures which I can only interpret as a gesture of encouragement to one whose scientific outlook was shaped in the Research Laboratories of this University. A little more than ten years ago when Professor Gopala Aiyar inaugurated a scheme for the detailed study of the brackish waters in and around Madras I was actively connected with this biological and faunistic survey. The results of this survey which have since been published[1] & [2] brought to the very forefront of our studies the problem of physiological adaptation in animals. While the majority of the marine animals at Madras would die even in moderate dilutions of sea water, how is it that a few of

[1] Panikkar, N. K. and Aiyar, R. G., 1937, 'The brackish water fauna of Madras' *Proc. Ind. Acad. Sci.*, Section B, Vol **6**, pp. 284-337.

[2] Idem, 1939 Breeding in brackish water animals of Madras. Ibid, Section B. Vol **9**, 343-364.

* These lectures were delivered while the author was Professor of Zoology at the University of Travancore.

them can survive, nay, thrive, in the constantly changing environments of the Adyar backwaters and how could some of the animals that normally live in the water remain exposed to air for long periods in the inter-tidal zone? It became apparent that an understanding of the physiological mechanisms in these animals was essential to analyse the processes at work in the evolution of their adaptive characters and the subject required altogether a new approach.

This new approach is something that not merely concerns the problems at Madras. It is of a much wider significance. The biological sciences at the present day are in a state of revaluation. Many of the older and accepted methods in biology have reached a saturation point in regard to their usefulness. Our subject has been essentially descriptive all these years and we have accumulated immense amount of data on the structure of living things. The '*form*' of animal life has been the chief occupation of the zoologist. The vast amount of information on the '*form*' acquired a new meaning with that great synthesis of biological thought of the last century, I mean the law of organic evolution. The next great step would be the correlation of '*form*' with '*function*' and it is in this direction that our subject is being reoriented. The most conspicuous progress in zoology during the past ten years has been in the field of '*function*', in the understanding of the living processes of animals. The result is the slow emergence of biology as an experimental science; the living animal has come to its own as against the museum specimen. Characters, instead of being described in the uncertain language of different qualities, are being assessed and quantitatively expressed by the methods of experiment, analysis and control. Even taxonomy is becoming more measurable and precise than merely descriptive resulting in what Julian Huxley rightly terms the "New Systematics." The sister sciences of chemistry and physics, which have for many years been unhelpful in the elucidation of biological phenomena, have in recent years made vast strides in what is their borderland that their aid in the study of biological problems is greater today than it was ever before.

The study of osmotic relations is one of those fields of investigations directed to the understanding of the living animal. Stated as a fundamental proposition it is the ability of the living matter to retain and regulate water and mineral substances essential for life without losing them to the surroundings.

HISTORICAL

The history of work on this aspect of animal physiology may be traced to the classical dictum of Claude Bernard in 1885: '*La fixité du milieu intérior est la condition de la vie libre*', i.e. the constancy of the internal medium is an essential condi-

tion for the existence of life.[1] Then followed the discoveries of Ringer. The direct study of the body fluids in relation to osmotic pressure may be said to begin with the important findings of Bottazzi early in this century when he made a series of freezing point determinations on the body fluids of animals at the Naples Marine Aquarium.[2] The fundamental difference between marine fishes (teleosts) and the marine invertebrates was discovered by him. He found also that whereas the body fluids of marine invertebrates and elasmobranchs have the same osmotic pressure as the surrounding sea water, the marine teleosts showed a concentration less than half that of the sea water. In recent years much progress has been made in our knowledge of osmotic changes in aquatic organisms, chiefly, in marine animals that can successfully withstand a certain amount of dilution of the external medium. Investigations by numerous workers have shown that the colonization of fresh and brackish waters by marine animals is closely connected with the development of osmoregulatory powers which are highly evolved in fresh water organisms. Schlieper and his school at Marburg[3] have done much to reinstate the interest in the subject by bringing evidence of the relative importance of the excretory organs in osmoregulation. Homer Smith and Keys gave the first physiological interpretation of the behaviour of the teleosts.[4] A discovery which has completely transformed our outlook on the subject is that of Krogh and his collaborators at Copenhagen in 1937 that animals in freshwater are capable of actively absorbing ions, more particularly chloride, from the surrounding medium, even when the latter ions are present only in exceedingly minute quantities.[5] This idea, an extension of the anion-atmung of Lundergardh in regard to plants,[6] has provided the explanation for many osmotic problems which till then remained unsolved. The whole subject is admirably reviewed by Krogh in his monograph published in 1939 at Cambridge.[7]

I should like to say a few words as to how I became interested in this subject. The need for finding physiological explanations on many problems of ecology encountered in the study of the Adyar Fauna has been mentioned already. When working in

[1] See Barcroft, J., 'Features in the Architecture of Physiological Function' (Cambridge, 1934) for an admirable essay on this topic with reference to the pH of body fluids. Also Dakin, W. J., Presidential Address, *Linn. Soc. New South Wales*, 1935.

[2] Bottazzi, F. 1897, *Archoiv. Ital. Biol*, 28, pp. 61-72. Idem 1908. *Ergebn. Physiol*, 7, 161-402.

[3] Schlieper, C. 1929, *Z. Vergl. Physiol.* **9**, 478-514; 1930 *Biol. Rev.* **5**, 309-56; 1935, *Biol. Rev.* **10**, 334-60. For other authors of the school of Schlieper consult Schlieper's 1935 review cited above.

[4] Smith, H. W. 1930. *Amer. J. Physiol.* **93**, 480-505; Keys, A.B. 1931. *Z. Vergl Physiol* **15**, 364-89, 1933 *Proc. Roy. Soc.*, B. **112**, 184-99.

[5] Krogh, A 1937, *Nature*, **139**, p 755, 1938 *Z. Vergl. Physiol.*, **25**, pp. 335-50

[6] Lundergardh, H 1937, *Bioch. Z.*, **290**, 104-24.

[7] Krogh, A, Osmotic Regulation in Aquatic Animals, Cambridge, 1939.

Britain early in 1939, I was inclined to expect any marine invertebrate to be isotonic with the surrounding medium; it was therefore of unusual interest to find two crustaceans in the sea water tanks of the Plymouth Marine Biological Laboratory showing a blood concentration markedly lower than that of the external medium. These are the palaemonid prawns belonging to the genera *Leander* and *Palaemonetes* about which I shall discuss at a later stage. But the interest aroused in the problem and its bearing on general biology have been so great that I have since then devoted the greater part of my studies to osmotic phenomena in marine organisms. The methods employed in this work and the fact that the problem impinges on aspects of biochemistry and biophysics enabled me to view these marine biological problems from a new angle. The fundamental importance of osmotic studies was also realised and, strange as it might seem, the osmotic problems tackled in relation to marine animals found parallel problems in totally unexpected branches of science like milk production and blood transfusion, subjects in which the exigencies of war made me interested.

LAWS OF OSMOSIS

It will probably be worth our while giving a few fundamental facts relating to osmosis. If two solutions differing in strength are separated by a semi-permeable membrane it is a well-known physical law that water will flow through the membrane until an equilibrium is restored; a similar movement of water will take place if one is pure water and the other a solution. The tendency for this movement of water sets up a certain pressure on the intervening membrane—a pressure which is spoken of as the osmotic pressure[1] &[2]. While in an ideal semi-permeable membrane the movement across is confined entirely to the solvent, many other biological membranes allow passage in varying degrees to different solutes; indeed there is hardly a biological membrane which is ideally semi-permeable. The rate of passage of water and salts through membranes will depend upon the difference in the strength of solutions they separate, the specific properties of the membranes and the temperature.

Of the physical laws governing osmosis the most important one which concerns the biologist is that the osmotic pressure of a solution varies directly with the concentration of the dissolved substance, the higher the concentration of a solution the higher its osmotic pressure. The volume of a solution is therefore inversely proportional to the concentration which would mean

[1] For a full treatment *vide* Glasstone · Text Book of Physical Chemistry, New York, 1940. Also Heilbrunn, L. V., An Outline of General Physiology. Philadelphia and London, 1943.

[2] A thermodynamical definition of osmotic pressure may be found in Lewis and Randall: Thermodynamics, New York, 1923.

that the well-known Boyle's Law of Gases which states that the pressure of a gas varies inversely as its volume is equally applicable to solutions. Likewise there is agreement with the Law of Charles since the osmotic pressure of a solution varies directly with the absolute temperature. The equilibrium that is reached finally will also depend on whether or not all the ions are diffusible. If only some of the ions are diffusible through the membrane the equilibrium may be unequal in regard to the specific distribution of ions on the two sides of the membrane, a condition that is usually spoken of as the Donnan equilibrium.[1]

METHODS OF STUDY

Many methods have been devised for the measurement of osmotic pressure of solutions. The earlier methods relied on direct observations of the rate of passage of salts and water through artificial semi-permeable of membranes. Pfeffer[2], the plant physiologist who first made satisfactory quantitative measurements of osmotic pressure on a sufficient number of concentrations, devised a type of osmo-meter in which natural and artificial membranes were used. The use of direct measurements of osmotic passage in biological work is, however, beset with numerous practical difficulties and experimental errors are often very high.

The method that is widely used by biologists for studying the osmotic pressure is the determination of the freezing point of the biological fluid concerned. Owing to the colligative properties of solutions there is a direct correlation between the freezing point and the osmotic pressure; the higher the osmotic pressure of a solution the lower its freezing point below zero. This method called Cryoscopy requires a minimum of about one ml. of a sample whose osmotic pressure is to be determined. This would be possible in the case of the larger animals which have an adequate amount of blood. With smaller animals the difficulties are obvious. Many of the invertebrates have at most a few drops of blood. The paucity of information on the composition of the body fluids of the lower animals has been mainly due to this and reliable data have been obtained only when techniques requiring only small samples have been perfected.

The most important development in the technique of osmotic pressure determinations has been brought about by the introduction of the thermoelectric vapour pressure method by A. V. Hill[3] in 1930. In essentials the method is to measure electrically the difference in temperature caused by difference in the rates

[1] In view of Willard Gibbs' earlier work leading to this concept this is more correctly termed the Gibbs-Donnan equilibrium.

[2] Pfeffer, W., 1877. Osmotische Untersuchungen, Leipzig.

[3] *Proc. Roy. Soc.* A., **127**, pp. 9-19, 1930.

of evaporation of two liquids of different vapour pressures. The solutions are placed in contact with thermojunctions arranged in a regular series in what is called the vapour pressure thermopile. The junctions are of two metals, silver and constantan. The changes in temperature cause an electro-motive force easily recorded as deflections from a sensitive moving coil galvanometer. Hill devised this method essentially to ascertain and study the heat production in muscles. But the great accuracy of this method for determining the vapour pressure of solutions was pointed out by him and the method has been successfully employed in osmotic pressure studies of biological fluids by Margaria, Fox, Bateman, Picken, Baldes, Beadle, and myself. It ought to be mentioned that even though the principle of the method remains the same, modifications in technique have been made by different workers, particularly Baldes.[1] In the original Hill instrument, a long row of thermojunctions exist on each side of the thermopile. On one side is placed a filter paper of standard size which is dipped in the unknown solution (blood) while on the other side is a similar filter paper dipped in a comparing solution, viz. a suitable solution of Sodium Chloride in water. The method is essentially a null-method, for, if the comparing and the unknown solutions have the same vapour pressure, the galvanometer will not show any deflection. The instrument is calibrated by using known solutions on both sides and the value for each mm. of deflection on the scale corresponding to the solution is obtained. On this basis the value of the unknown is calculated.[2]

In Baldes' modification of the thermoelectric method, a sensitive thermocouple replaces the thermopile, and because of the simplicity of construction and the greater efficiency of the thermocouple this modified method has been found more useful in the study of osmotic problems in biology. The amount of body fluids or blood required for making a single estimation is also very much smaller. A single tiny droplet of a sample is enough to make a satisfactory determination. This method has been widely used by me in my studies on different animals, mainly the Crustacea.

The solutions are mounted on the thermocouple in a humidified box to prevent evaporation causing errors in estimation. The thermocouple in its thermopile chamber was equilibrated in a thermostat kept constant at 20°C. ±0.001, and the deflections taken from a Downing moving coil Galvanometer of sensitivity 1.28×10^{-8} volt per mm. at a distance of 3 meters. Using tested thermocouples, one mm. deflection on the scale was equivalent

[1] *J. Sci. Instr.*, Vol. 11, p. 223, 1934.
[2] For full discussion of the theory of the method *vide* Hill, A. V., *Adventures in Biophysics*, Oxford, 1931. Also Baldes, E. J. *Biodynamica*, 1939.

to an E.M.F caused by solutions differing in strength by 0.005% NaCl.[1]

THE PROTOZOA

With this brief introduction on methods we will review the osmotic behaviour in the animal series beginning with the very simple condition shown by Amoeba and other Protozoa. In Amoeba the body, which is made up of a single cell, is not provided with any special covering membrane but the ectoplasm serves as the region which regulates the passage of water and substances in and out. It is obvious that the living substance of Amoeba cannot carry out the normal physiological activities were its protoplasm as dilute or as deficient in salts as the surrounding fresh water. The protoplasmic body being of a higher osmotic concentration than fresh water, there is a constant stream of water from the surroundings into the body of the Amoeba. It is this water which is periodically expelled by the contractile vacuole. The general belief is that the function of the contractile vacuole is excretory but its main function is in osmotic regulation. There are species of Amoeba which could be acclimatized to sea water but the contractile vacuole disappears when Amoebae are reared in saline media. The absence of contractile vacuoles in haemoflagellates and Sporozoa may be explained by the fact that these organisms live in more or less isotonic surroundings.

I may mention, in this connection, some of the views put forward by cytologists on the properties of the membrane surrounding the contractile vacuoles. This membrane is osmiophilic in character and is darkened by osmic impregnation methods showing a very similar reaction as shown by the Golgi bodies. This has led Gatenby[2] to suggest that the function of the Golgi bodies, at least in the protozoa, is to act as a dehydrating organ to collect and accumulate water into the contractile vacuole. If this view be further substantiated, which it no doubt needs, the Golgi bodies would seem to have an osmo-regulatory function at least in the Protozoa.

There are many ciliates capable of living in fresh, brackish and sea water. Among them there is difference in the rate of activity of the contractile vacuoles. In a painstaking study Kitching[3] has brought out the correlation between the vacuolar output and the salinity of the medium. To this observation may be added that marine ciliates are, as a rule, without contractile vacuoles whereas all freshwater once have them.

Direct determinations on the osmotic pressure of protozoa are not easy owing to their very small size. The plasmolytic

[1] Panikkar, N. K., *J. Mar. Biol. Assoc.*, Vol. **25**, p. 320, 1941.
[2] Essays on evolution presented to E. S. Goodrich, 1939, Oxford.
[3] Kitching, J. A., The physiology of the contractile vacuoles, Parts I to III, *J. Exp. Biol.*, Vols. **11**, **13** & **15**, 1934-38.

methods of study adopted in plant cells are not applicable to protozoa as they are most delicate and are not easily maintained under experimental conditions. Picken[1] tried to determine the osmotic value by making centrifuged concentrates of *Spirostomum* and found this protoplasmic jelly to be in the neighbourhood of 0.085% NaCl (25 mM). This value cannot be taken with any finality as it would have been difficult to have removed completely the water in between the organisms but it is worth mentioning that this value is fairly close to that arrived at by Kamada[2] from a study of the vacuolar output in *Paramecium caudatum*.

A most interesting material for osmoregulatory studies on Protozoa is *Noctiluca* the Dinoflagellate which is well known for its luminosity and occurs in enormous swarms near tropical coasts.[3] This organism is of a much lower specific gravity than sea water as it can float in fairly low dilutions of sea water and its cell wall is definitely permeable to water. Goethard and Hensius[4] made an excellent experimental study and found that the lower specific gravity and at the same time osmotic equilibrium of *Noctiluca* with sea water can only be by the presence in its protoplasm of some osmotically active substances of definitely lower specific gravity than NaCl. By a series of tests they showed it to be ammonium chloride. The significance of this discovery lies in the fact that the ionic composition of the cell sap would then be entirely different from outside sea water, *Noctiluca* thus showing an advanced instance of ionic regulation even among unicellular organisms.

THE PORIFERA

Although predominantly marine, the sponges include several species inhabiting freshwater and brackish water. These are, however, confined to certain families. Owing to the primitive organization of the sponges, the regulatory function would probably be individually discharged by the component cells of the sponge tissue. There has been much confusion in the literature whether or not contractile vacuoles exist in the collar cells of fresh water *Spongilla*, while some of the early workers on sponges have indicated the presence of contractile vacuoles even in marine species.[5]

[1] Picken, L. E. R., *J. Exp. Biol.*, 13, 1936.

[2] Kamada, T., *J. Fac. Sci. Univ.*, Tokyo, Vols. 4 & 5, 1935-36.

[3] In Madras, swarms of Noctilucae are observed on the coast during July to September.

[4] This work was done in 1892 but published only in some inaccessible official report. A summary may be found in Krogh's (1939), monograph.

[5] In a recent study Jepps (*Proc. Roy. Soc.*, *B* 134, pp. 409-417) has cleared up this question. She has shown that contractile vacuoles are definitely present in both amoebocytes and choanocytes of freshwater sponges while they have not been seen in the cells of several marine species found on the British Coasts.

THE COELENTERATA

In Coelenterata we do not come across any great powers of regulation. Among the marine forms there is practically no difference between the external medium and the fluid contained in the gastrovascular space. This is equally true of fresh water species where the coelenteron presumably contains fresh water. The regulation, if at all existing, would be resident in the ectoderm and endoderm. While the group is predominantly marine there are only a very small number of fresh water representatives and unfortunately we have no data on the osmotic behaviour of these fresh water forms. Palmhert[1] found that fresh water polyps like *Pelmatohydra* and *Chlorohydra* could stand concentrations of salt up to 2–2·5‰ while the brackish water *Clava* could be acclimatized to 10–30‰. The evidence is that the osmotic concentrations of fresh water coelenterates is extremely low. The marine jelly-fish have been found to have much the same total concentration as their surroundings. The Coelenterata would probably offer the most suitable material for the study of regulation of tissues and cells as there are quite a few species of marine origin inhabiting brackish waters. Among the Ctenophores there are no fresh water or even brackish water species.[2]

THE PLATYHELMINTHES

The flat worms include marine, as well as fresh water representatives. The principal osmoregulatory organ would seem to be the flame cell functioning in much the same way as the contractile vacuole of protozoa.

There is a marine triclad, *Gunda ulvae*,[3] on which some interesting results have been obtained and consequently it needs special mention. This organism is a common inhabitant of the estuaries of Great Britain. The animal is able to thrive apparently being unaffected by changes in the external medium which is practically fresh water during low tide and practically sea water at high water mark. Pantin[4] made a careful study of the changes taking place in this animal under experimental conditions. When worms from sea water are transferred to fresh water there is an immediate swelling of the body and conspicuous increase in volume. But this increase in volume is slowly

[1] *Zool. Jahrb. Abt. Allg. Zool.*, 53, 1933.

[2] There are many Sea Anemones that live in brackish waters, *vide* Panikkar, N. K., Proc. Zool. Soc., London, 1936 & 1939. *Zool Jahrb. Abt. Anat.*, Bd. 63, 1937 and Panikkar and Aiyar, *op. cit.*

[3] According to the strict application of rules of nomenclature this should be *Procerodes ulvae*.

[4] Pantin, C. F. A., Adaptation of Gunda ulvae to salinity, Pts. I to III —*J. Exp. Biol.*, 8, pp. 63-72 & 82-94, 1931 and Weil, E. and Pantin, C. F. A., Ibid, pp. 73-81, 1931.

regulated after a while and the animal returns to normal. The work was continued by Beadle[1] who demonstrated the presence of vacuoles in the intestinal cells of animals kept in fresh water. Adaptation would appear to be in a large measure the result of activity of these cells which can receive and separate the excess water without causing damage to the tissues.

It was found by Pantin that the animals in their natural environment could survive a much greater variation in salinity than those kept at the laboratory where dilutions of sea water were made with Plymouth tap water. A series of controlled experiments enabled him to point out that the presence of Calcium ions is essential for the worms to survive these environmental changes. Pantin found that the amount of Calcium in sea water and in the stream where *Gunda* were found in large numbers was comparatively high whereas the Plymouth tap water being very deficient in Calcium was an unsuitable medium for dilution.

This subject has been mentioned at length because the presence of Calcium is now admitted as a most important factor in the osmoregulation of lower invertebrates.[2] There is possibly no other ion which gives so specific an effect as Calcium. The exact effect seems to be that (*a*) Calcium is essential for the stability of mucus which plays a very important part in the insulation of animal membranes and (*b*) the fact that Calcium is essential for the maintenance of the intracellular matrix of living cells. From the work of Herbst[3] it is now known that the blastomeres of developing sea urchins would separate without being held together if the Calcium ion is not present in the surrounding medium. Lastly, the phenomenon of salt antagonisms discovered by Loeb and confirmed by many subsequent investigators would offer a relevant explanation. Even though sodium and chlorine ions form the major constituents of sea water it is not so well known that Sodium chloride by itself has a toxic effect upon living cells. Potassium, though an essential constituent of protoplasm, is indeed, a toxic substance by itself. But the striking fact is that the Calcium ion is able to neutralise the toxic effects of the other metallic ions. This important contribution now known as salt antagonisms is universally accepted in physiological work. Its practical utility was known long before Loeb made this discovery. As far back as 1882, Ringer found that if he desired to keep an isolated frog's heart beating it was disastrous if he used ordinary saline solution, even though this solution is prepared isotonic with frog's blood.[4] He

[1] Beadle, L. C, 'Osmotic regulation in *Gunda ulvae*' *J. Exp. Biol.*, **11**, pp 382-96, 1934.

[2] For a critical review of this subject *vide* Robertson, J. D. *Biol. Rev.* Cam., **16**, 1941 and Heilbrunn, L. V., *An outline of General Physiology*, Philadelphia, 1943.

[3] *Arch. Entwicklungesmech.* **9**, 1900.

[4] For these and subsequent work *vide* Ringer and Sainsbury *J. Physiol.* **16**, 1894.

tried adding other substances to the normal saline in order to make it harmless and found that the white of egg was admirable for this purpose. Further analysis led him to discover that if the Sodium chloride, solution be mixed with a small and appropriate amount of Calcium chloride, Potassium chloride and a trace of Sodium carbonate, it would be possible to keep the isolated tissues alive and active in this solution. This solution, of course, is known as the Ringer's solution and it is an interesting fact that the relative proportions of Sodium, Potassium and Calcium in it are very close to the proportion of the same salts in sea water.

There is very little information on the osmotic properties of the parasitic flat worms. The Cestodes and Trematodes which inhabit the alimentary canal of vertebrates live in environments subject to a great amount of variation. Further, they seem to be immune to the effect of gastric and intestinal secretions. The fact that they thus live in everchanging media, often charged with secretions, has given rise to the popular belief that the body wall, particularly the cuticle of parasitic flat worms and Nematodes is impermeable to water and salts. For example, even as recently as 1939, we find Krogh[1] mentioning that the cuticle of parasitic worms is impermeable to water and salts.

THE NEMATODA

The lead in the study of the body fluids of parasitic worms was given by the Swiss physiologist Schopfer[2] who in 1932 made a series of freezing point determinations on the body fluids of *Ascaris* from the pig, the horse, the ox and the humans. Schopfer found that the osmotic concentration of *Ascaris* from mammals was in the neighbourhood of 1·2 to 1·4% NaCl or Δ — 0.62 to 0.78°C in external media of—0.75—1.0°C. He also found that when worms were transferred to distilled water there was a considerable amount of inward passage of water. As a matter of fact, the permeable nature of ascarid cuticle was anticipated by Schopfer. Unlike the ascarids, Schopfer found that *Proleptus obtusus* living in the alimentary canal of marine elasmobranchs showed a Δ of -2.55°C in an external medium of Δ —2.40°C.

Very definite evidence that both water and salts could pass through the body wall of parasitic nematodes has been brought forward by Sproston and myself.[3] We investigated the Ascarid, *Angusticaecum*, which is a parasite of *Testudo graeca*. The worms are capable of surviving in distilled water and tap water for considerable periods outside their host. In tap water a more or less steady value of 1% NaCl is maintained, but there

[1] Krogh's 1939 Monograph, *op cit*
[2] Schopfer, W. H , *Rev Swisse, Zool* , 39, pp. 59-114, 1932.
[3] Panikkar, N K & Sproston, N. G., Osmatic relations of some metazoan parasites. *Parasitology*, Cambridge, 33, pp. 214-23, 1941.

is a certain amount of diminution in osmotic values in distilled water. From ligaturing experiments we found that water passes in and that salts from the body of the worm escapes to the exterior. These were confirmed by direct measurements of the osmotic pressure of normal, experimental and ligated worms. There does not seem to be any ground to justify the assumption that the cuticle of nematodes is impermeable.

This conclusion is supported by evidence on a free living soil nematode, *Rhabditis terrestris,* which has been investigated recently by Stephenson.[1] He showed that the main aqueous exchanges occur through the cuticle and protoplasm covering the pheriphery of the body and are controlled by the protoplasm rather than by the cuticle. The species normally lives in an environment which is hypotonic to the body fluids and maintains its internal osmotic pressure by means of an active method in which the alimentary canal plays a part. In concentrated solutions, water is removed from the body and the internal osmotic pressure may also be increased to some extent by the penetration of osmotically active substances to the body through the cuticle and the hypodermis of the body wall. During recovery in concentrated solutions, there is a slow removal of salts from the body. In distilled water swelling occurs because the initial entry of water is too great to be coped with by the existing powers of regulation.

THE POLYCHAETA

The annelids have formed a popular subject of study among many recent workers. The polychaetes are a group essentially marine but a few species invade brackish water and there are many estuarine species capable of living in diluted sea water. There are two species of *Nereis,* similar in appearance but very different in physiological reactions. *Nereis diversicolor* is an estuarine form. It can withstand dilution of the environment up to about 30% sea water. *Nereis (perinereis) cultrifera* on the other hand, will die almost immediately if the surrounding medium were below about 70% sea water.

One convenient way of analysis of osmotic changes taking place when animals are transferred from one medium to another is to examine the changes in weight taking place in successive periods after transfer. When an animal is taken from sea water to dilute sea water, the passage of water from outside into the body of the animal will be very much in evidence immediately after transfer. There is swelling of the animal owing to increased water content. It will naturally take some time for the animal to attain equilibrium with the surroundings. So in this case, there is an initial gain in weight and later return to normal. The

[1] Stephenson, W., Effect of variation in osmotic pressure upon a free living soil nematode. *Parasitology,* 34, pp. 253-65, 1942.

amount of increase in weight, the time taken for the final equilibrium to be restored and the maximum weight indicated are suitable standards to compare the permeability and the regulatory mechanism effective in the animal. A necessary corollary would be to check this with direct measurement of the osmotic properties of the body fluids at corresponding periods.

The fact that *Nereis* could be handled easily has been taken advantage of by workers like Beadle[1] who have carefully studied the weight changes in *Nereis diversicolor* and *Perinereis cultrifera.* It would appear that the mechanism of effecting quick final equilibrium is much more efficient in *Nereis diversicolor* and this, no doubt, would account for its adaptability. But more important is the fact that this species is able to maintain the concentration of its body fluid slightly higher than the surroundings in whatever concentration of dilute sea water. In other words this species is euryhaline unlike *Perinereis cultrifera* which is stenohaline and would show an identical concentration with the surroundings. Beadle showed this by direct measurements of osmotic pressure. But an indirect method was employed by Ellis[2] who found that the chloride output of the two worms in distilled water or in dilute sea water was different, and made the suggestion that the weight regulation of the worms is not accompanied by osmoregulation. Ellis also indicated that physiological differences in the same species from different habitats may occur as he found from the behaviour of worms from Roscoff and from Plymouth.

The question of adaptation of polychaete tissues to hypotonic environments was taken up by Wells and Ledingham[3] who made a series of careful experiments on the reaction of isolated rhythmic preparations of *Arenicola marina, Nereis diversicolor,* and *Perinereis cultrifera.* Their results showed that during the transfer of tissues from sea water to a hypotonic fluid, there is a brief excitement followed by a complete inhibition and finally a gradual return to activity corresponding to the accommodation to the new medium. They also found that the shock effects of rapid changes are unlikely to be evoked under natural conditions.

A point to be emphasised in all these experiments is that the degree and the slowness with which transfer is effected from one medium to another is a matter deserving attention. There are many forms in which direct transfer would be fatal but by slow acclimatization it is possible to bring down the saline concentration of the medium without apparent ill-effect to some of the organisms. It is here, that the euryhaline habit of *Nereis*

[1] Beadle, L. C., *J Exp Biol.*, **8**, 1931 and *J. Exp Biol*, **14**, 1937
[2] Ellis, W G, *J Exp Biol*, **14**, 1937.
[3] Wells, G P. and Ledingham, I. C., Physiological effects of a hypotonic environment I *J. Exp. Biol*, **17**, pp 337-352, 1940.

diversicolor is to be contrasted with the behaviour of burrowing polychaetes like *Arenicola*. Schlieper[1] found this animal in regions of salinity as low as 8 per mille. But experimental work with *Arenicola* shows that it is not capable of maintaining a body fluid concentration hypertonic to the external medium. The general conclusion that could be arrived at in regard to *Arenicola* is that this animal is without any powers of regulation but that it can maintain metabolic activity in media ranging from sea water up to considerable dilutions of sea water. It is probable that the wide range of tolerance shown by *Arenicola* where active regulation is absent is shared by many other polychaetes.

There is strong evidence that the nephridia play an important role in the osmoregulation of polychaetes. Ewer and Ewer[2] made a study of the weight changes consequent on transfer of *Sabella pavonina* from sea water to 75% sea water using amputated worms. They found that regulation is evident in portions of the worms with the large thoracic nephridia.

THE OLIGOCHAETA

In sharp contrast with the polychaetes are the earthworms. This is an essentially fresh water group, even though the majority of the members of this group have taken to a terrestrial life. The aquatic nature of their origin need not be doubted when we consider for a moment the fact that a certain amount of moisture in the surroundings is essential for their life. Considering how the earthworms are most common and easily obtained it is regrettable that our knowledge of their regulation is most inadequate.

I have found the body fluid concentration of the common earthworm in Britain to be in the neighbourhood of 1% NaCl. Maluf has recently made a study of the regulation in the American earthworms.[3] His values are similar to mine but he found considerable range of variation among individuals of the same species, this, no doubt, being correlated with their water content. Earthworms could be kept in fresh water for many weeks but after the initial reduction in internal osmotic pressure a more or less steady value is maintained. He visualises that there is a certain amount of ion absorption both from the general body surface and from the alimentary canal, a conclusion which is supported by the difference in their behaviour in fresh water and distilled water. Recently, Stephenson[4] has brought interesting evidence to show that earthworms are capable of

1 Schlieper, C., *Zeit. Verg. Physiol.*, **9**, pp. 478-514, 1929.
2 Ewer, D. W and Ewer, R. F. 1943 *Nature*, Lond., **152**, pp. 598-599.
3 Maluf, N S. R., *J. Cell Comp. Physiol.* 1941.
4 Stephenson, W., *Nature*, London 155, p 635, 1945.

hypotonic regulation as judged by measurements on *Lumbricus* acclimatized to saline media.

Owing to their terrestrial habits the osmoregulation in earthworms is closely linked up with the problem of water regulation. The majority of the earthworms thrive only if there is plentiful supply of water in the soil. But in India, there are exceptions like *Pheretima.* This worm is capable of living in soil that contains very small amounts of water. Bahl[1] who for many years has been studying the structure and behaviour of *Pheretima* has been able to correlate this habit with certain anatomical peculiarities. The excretory organs in earthworms are, as a rule, the paired nephridia which open to the exterior by means of a series of paired apertures. In *Pheretima* and certain other Indian earthworms the nephridia, instead of opening to the exterior, discharge their contents into the gut by means of connections from two lateral excretory vessels. The gut which receives the nephridial contents seems to be capable of reabsorbing the water as evidenced by the very low water content of the faecal pellets. Bahl has given a comparison of *Pheretima* with another north Indian worm *Eutyphoeus.* In the latter the nephridia are not enteronephridial, the moisture content of the castings is extraordinarily high and this is in full agreement with its habits of life as it is found only in situations with plentiful supply of water.

Like true fresh water species it may be expected that the nephridia play an important role in the discharge of excess water and there is striking confirmation of this from the measurements made but as yet unpublished by Bahl.[2] He found the freezing point depressions of the urine of *Pheretima* to be about $-0.065°C$ while its blood and coelomic fluid showed values $-0.40°C$ and $0.285°C$ respectively.

THE MOLLUSCA

The Mollusca are again a predominantly marine group. The number of species which are found in fresh and brackish water forms only a small part of the total comprising the phylum. Bottazzi and Monti made measurements of the blood of marine Molluscs like *Aplysia, Cassis, Ostrea, Mytilus* and *Octopus* and found these animals to be isotonic with the surrounding medium.[3] There are many species of marine molluscs, especially in Indian waters, that can tolerate brackish water. There is no information on the mechanism of their adaptation. It is likely that these are also euryhaline even though only to a limited extent.

[1] Bahl, K. N *Quart. Journ. Micr* 76, 1934.
[2] I am very grateful to Prof Bahl for allowing me to quote these figures. Since writing this, Bahl's results have been published in *Quart. J. Micr. Sci.,* **85,** pp 343-389, 1945.
[3] Bottazzi, *Op. cit.*

When dealing with molluscs, one has to bear in mind that since they are provided with protective shell, it is possible for them to withdraw into their shells in unfavourable environments. In very dilute sea water even euryhaline molluscs will completely retract, complete adjustment with the surrounding medium being brought about only many days hence.

The osmotic properties of fresh water molluscs have been examined by a number of investigators but the recent and most useful figures are those given by Picken.[1] By means of the vapour pressure method he found that the blood concentration of *Anodonta* is very low with an average value of 16.2 mM and that the pericardial fluid had more or less the same molar concentration as blood. Slightly lower values have been obtained in the case of *Limnaea*. In *Anodonta* Florkin[2] took samples of urine near the pericardium and found that the values agreed fairly closely with that of blood whereas the final urine eliminated is very much lower than blood in total concentration. There is strong evidence therefore that active reabsorption of salts must be taking place in the nephridial canals.

The low osmotic values presented by the fresh water mussel must be taken in conjunction with the remarkable behaviour of many land snails and slugs. Howes and Wells[3] made a study of the changes in the water content of *Helix* and *Arion* and found that the water content is subject to great variation. It is possible to keep animals soaked in water and induce a higher water content in their living tissues. Similarly, the snails and slugs which have been exposed to air for long periods indicated much loss of water from them. The variations may be as great as 30% of the total weight. This is not surprising considering the habits of snails and slugs. But what is a matter of real interest is that the blood concentration in these animals present the same fluctuation as does the water content. When Duval[4] made a number of measurements in 1915 on the osmotic pressure of the blood of snails, he was struck by the enormous variations in the values for blood and he made the interesting finding that whereas they varied between −0.37°C and −0.43°C in hibernating animals, active summer snails showed only values between −0.30°C and −0.4°C. The results of Duval have been confirmed by Kamada[5] using the Hill method according to whom the hibernating blood is isotonic with 0.69% NaCl and the active with 0·50% NaCl.

[1] Picken, L. E. R., Excretory mechanism in certain Mollusca. *J. Exp. Biol.*, **14**, 1937.

[2] Florkin, M., *Bull. Acad. Belg. Cl. Sci.*, 1935, **432-5**.

[3] Howes, N. H. & Wells, G. P., 'The water relations of Snails and Slugs', *J. Exp. Biol.*, **11**, 1934.

[4] Duval, M., *Ann. Physiol. Phys Chem. Biol.*, **6**, 1930, p. 346.

[5] Kamada, T., *J. Exp. Biol.*, **10**, 1933, pp. 75-8.

REGULATION IN ARTHROPODA

In Arthropods, owing to the haemocoelic nature of the body cavity there is no distinction between blood and coelomic fluids. Because of this, the Arthropods, more particularly the Crustacea, have been a very favourite material for the study of problems in adaptation.

In the animals that we have hitherto dealt with the body wall, being soft, has been more or less freely permeable to substances, both water and salts. It is only in instances like the mollusca that external protective devices could be brought into operation. In the whole group of Arthropods, the development of the chitinous exoskeleton has brought new problems of permeability. The valuable work of Yonge[1] on the nature and permeability of chitin has solved many of the difficulties presented in this field of study. He demonstrated by microchemical and histological tests that the chitin of Arthropods is actually permeable to water and salts and that it is the cuticle occurring as a uniform covering on the outside of chitin which forms the impermeable layer. This substance called cutin is produced by the cells of the ectodermal epithelium and spreads over the chitin as it is a substance of exceptionally low surface tension.

THE CRUSTACEA

The majority of the Crustacea are marine. It is only a comparatively few of the crustaceans that have secured a foothold in fresh water and land. Numerous studies have been made on marine Crustacea. Animals like the marine lobster, crabs and prawns are stenohaline and are unable to survive dilutions of sea water. They have the same osmotic pressure as sea water of their normal environment. But when these animals are immersed in dilutions of sea water, many of them show almost an isotonic osmotic curve. There is a considerable degree of swelling and few stenohaline species could tolerate a change of more than about three parts per thousand of salt concentration.

A good number of the shore-living and littoral Crustacea are, however, able to tolerate dilutions in their environment. Especially, in the tropics, where the rainfall is heavy and where large rivers open into the sea, the coastal salinities are appreciably altered and it is to be expected that these shore-living forms will show some adaptive power that is denied to the more typical marine members of the group. Consequently, many shore-living tropical Crustaceans are euryhaline and among the Decapods a number of species have invaded brackish and fresh water and have also taken to the terrestrial habitat directly from the sea.

[1] Yonge, C. M., *Proc. Roy. Soc.*, B, **111** & **121**, 1932 & 1936. *Proc. Zool. Soc. London.*, **107**, 1938.

A well known euryhaline crab of the European coasts is *Carcinus maenas* which has formed the subject of several studies by Schlieper, Sholles, Schwabe, Bateman, Hukuda, Picken and Webb.[1] This shore crab is isotonic in sea water and its urine is isotonic with blood. It is able to live in media of salinities as low as 10 parts per mille. Its osmotic behaviour shows a remarkable capacity to maintain a fairly higher value than the medium when the animal is placed in dilute sea water. In other words, the animal has exceptional powers of what may be termed hypertonic regulation. How is the regulation achieved? The permeability of the gills of the species is unquestionable. The output of chloride when the animal is placed in fresh water is also considerable. The rate of urine production is, no doubt, increased to begin with but the urine is isotonic with blood whatever the medium. It was evident even from very early work that osmoregulation is achieved by an active living process as postulated by Hill, Margaria and Bateman. But what this active process was remained unsolved until Krogh's idea of active ion transport was formulated.

Comparative studies on the rate of change in weight of marine animals including *Carcinus* was made by Hukuda[2] who found that the time co-efficient for *Carcinus* was different from that for stenohaline marine crustacea. There was no doubt, a low degree of permeability in *Carcinus* as compared with other crabs like *Hyas*. The general regulation in *Carcinus* would therefore appear to be the low permeability of the integument on the one hand and the active uptake of salts on the other.

MOULTING AND OSMOTIC CHANGES

A complication introduced in Crustacea is that the animals moult at several times during their lifetime, a feature that is common to all Arthropods. Immediately after moulting, the Crustaceans absorb a considerable quantity of water into their body as shown by the changes in water content of Crustaceans immediately after moult and during intermoult periods. The water absorbed is through the integument which is soft and highly permeable in the newly moulted crab or prawn.

Interesting osmotic changes in correlation with the moult cycle have been discovered by Baumberger and Olmsted[3] in *Pachygrapsus* where a notable increase in the osmotic concentration of the blood before moulting has been suggested as the factor which gives sufficient osmotic force to the water to be absorbed immediately after moult. I have found a similar

[1] Webb, D. A. Ionic Regulation in *Carcinus maenas*. *Proc. Roy. Soc.*, B. **129**, 1941. The earlier references to work on *Carcinus* are cited in this paper.
[2] Hukuda, K., *J. Exp. Biol*, **9**, 1932.
[3] Baumberger, J. P. and Olmsted, J. M. D., *Physiol. Zool.* **1**, 1928.

increase in osmotic concentration before moult and later return to normal in the Palaemonid prawns.[1]

It is noteworthy in this connection that Robertson[2] working on *Carcinus*, found that a Crab having a fresh weight of 50 g. would absorb 35 g. of water during moult. The corresponding figures for *Maia squinado*, *Pachygrapsus crassipes* and *Hemigrapsus oregonensis* are 58, 17 and 22 g. respectively, while the corresponding value for *Leander serratus* is only 15.7 g. Lowndes and I[3] have found that *Homarus* of fresh weight 100 g. absorbs about 47 g. of water within less than 2 days after moulting. The difference between the amount of water absorbed by the stenohaline *Maia* and euryhaline *Carcinus*, and the much lower value for *Pachygrapsus crassipes* and *Leander serratus* would seem to be closely related to the differences in the permeability of the integument of these crustaceans.

(*The Second Part of the Lectures will appear in a later issue of this Journal.*)

[1] Panikkar, N. K., *J Mar. Biol. Assoc.*, **25**, 1941.
[2] Robertson, J D., *Proc. Roy. Soc.*, B. 126, 1937.
[3] Lowndes, A. G & Panikkar, N K., 'A note on the changes in the water content of the lobster *Homarus vulgaris* M. Edw. during moult' *J. Mar Biol. Assoc.*, **25**, 1941.

SEX DIFFERENCES IN FOUR GENERA OF COPEPODS PARASITIC ON INDIAN FISHES

BY

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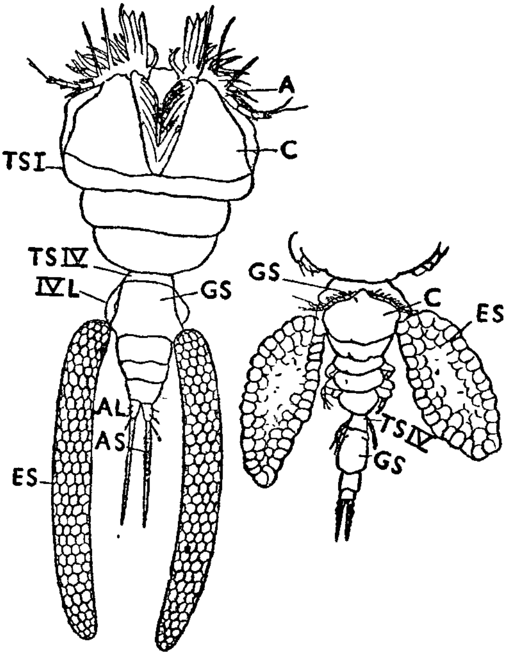
The marked difference in the sexratio among Copepods always arrested attention. As in the case of the Amphipods studied by Segestrale, the comparative scarcity of the male is to be explained by their earlier maturity, their not feeding during the adult period and their dying soon after the first breeding season. This



1*a*. *Bomolochus* sp. female
Fig. 1*b*. *Bomolochus* sp. male attached to body of female, between her egg sacs

A = Antenna I
AL = Anal lamena
AS = Anal setae
C = Carapace
ES = Egg sac
GS = Genital segment
IV L = Fourth leg
TS IV = Fourth thoracic segment

difference in sexratio is so marked among parasitic Copepods that in the case of several Ergasilids in which the adult females possess the power of leaving one host for another, the adult males

are so scarce as to be 1 in 200. On the basis of this and the fact that several larval males are noticed metamorphosing into adult, specialists like Wilson have generalised that it is probable that males die after copulating with females which receive sperms enough to fertilise all the eggs they will ever lay. Therefore it is of interest to record the finding of the male *Bomolochus multispinosa* attached to a female already bearing eggs. It is significant that in the Caligids the difference in numbers is not so great and several males of these skin and gill parasites can be found attached to the same host and the males are not particularly suited for natatory mode of life. This difference in ratio is lessened in Dichelesthids, where the males are as helpless as the females, and equally degenerated from the Copepods level and remain attached throughout life to the host. In the case of the Lernaeopodids which are more degenerate, the sexes are equal numerically—every female having a male[1] attached to its cephalothorax.

DIFFERENCE IN SIZE

In the less modified Caligidae like *Caligus polycanthi* the female is nearly of the same size except in the width of the genital segment and the additional length gained by the egg strings (vide figs.). In *Bomolochus multispinosa* where the male continues to be attached, presumably long after the copulation i.e. even when the egg strings have been formed, the male is less than half the length, being 1.36 mm., including the anal setae, while the female is 2.75 mm. In *Lernanthropus dussumieria* the females measure about 2.6 mm. in length, while the males are only 1.5 mm. long. But in the still more degenerate Lernaeopodid, *Clavellisa dussumieria,* the male is only a twelfth of the female (being only .16 mm. long) and is found clinging to the neck-like Cephalothorax of the female (which is 2 mm. long).

SEX DIMORPHISM IN *Caligus polycanthi*

The male is slightly smaller measuring 3.68 mm. in length (while the female is 4 mm. long) but appears to have a more slender and long body. This is because the carapace is slightly longer being 2.19 mm. as against 2 mm. in the female and also because the genital segment is comparatively smaller in the male than in the female. The posterior part of the carapace being as wide as the length of the carapace, the outline of the cephalothorax is distinctly different from that of the female. The persistence of the vestiges of the 5th as well as the 6th appendages while only those of the former are found in the female, indicate a more primitive condition. The second antennae are longer, more adapted for prehension as also the first maxilla and the second maxillipede. The abdomen is better developed than in the female where the

growth of the long egg sacs must alter the conditions necessary for their support, inhibiting the free play of the anal setae. The widening and lengthening of the genital segment in the female, precocious in the larva, is directly related in the adult, with the formation of the eggs.

Sex Dimorphism in *Lernanthropus Dussumieria*

The male has a dorsal shield or carapace which is roughly hexagonal in form while in the female this is sub-quadrangular.

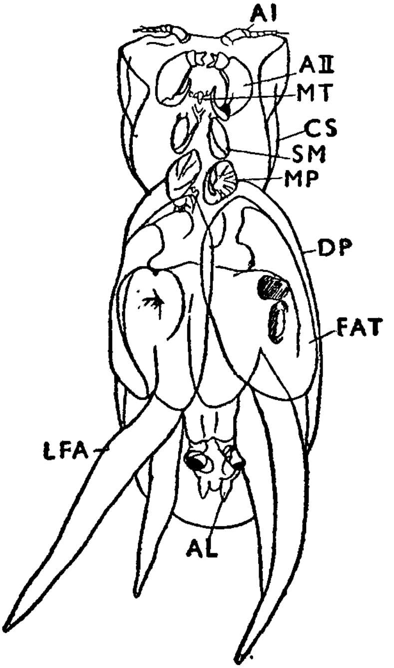


Fig. 2a. *Lernanthropus Dussumieria* female

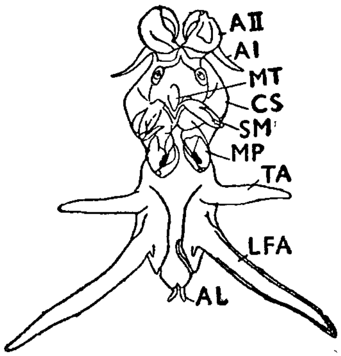


Fig. 2b. *Lernanthropus Dussumieria* male

AI = First Antenna
AII = Second antenna
AL = Anal laminae
CS = Cephalic shield
DP = Dorsal plate
FAT = Foliaceous appendage of third segment
LFA = Laminae of fourth appendage
MP = Maxillipede
MT = Mouth tube
SM = Second maxillae

In the male, there is no dorsal plate such as covers the rest of the body and appendages in the female. The second antennae are larger and capable of being swung forwards as also the second maxillae and maxillipedes. The first two thoracic appendages show several differences from those of the female. These however like the uniramous character and cylindrical form of the next two succeeding appendages (vide Figs.) are not based on any functions peculiar to the male and therefore must be explained as the result of the difference in sexual metabolism.

Sex Differences in *Bomolochus multispinosa*

In the male the body is much more slender being only 0.36 mm. broad at the cephalothorax. Not only are the six segments

of the thorax distinct (as probably in no other parasitic form) but even the segment which had fused with the cephalon to form the cephalothorax appears indicated by a faint groove. The fourth segment is only half as broad as the first, marks a waist more distinct than the female. The weakly plumose character of the pointed setae of the first antenna as well as the clinging nature of the bristles of the distal part of the same appendage, are less suited for swimming than in the female. The mandibles and the maxillae are as in the female. But the maxillipedes are proportionally far larger. It is the chief organ of prehension, its claws being suited to hold on to the groove in front of the genital segment of the female (vide Figs.). The thoracic appendages are of the same natatory character but of weaker build. When these various features are collectively considered, the fitness of the male for a free swimming mode of life may be doubted. The vestiges of the 6th leg present in the female being absent in the male, makes a challenging contrast to the retention of a more primitive segmentation by the male.

Sexual Dimorphism in *Clavellisa Dussumieria*

In the sub-family *Clavellinae* as in the family Lernaepodidae as a whole, the copepodid characters are entirely lost in the adult stage. The male is a very diminutive creature, ovoid in form, attached to the middle of the cephalothorax of the female by the maxillipedes whose tips are pushed below the surface level and aided by the two hooks at the end of 2nd maxillae. The rest of the appendages are directed forwards. The body which is without any trace of segmentation, is covered by a dorsal convex shield or carapace and is flexed above the posterior two-thirds of the length. Even the anal laminae and vestiges of the limbs seen in the female are absent.

Incidence of Sex Differences in *Caligus polycanthi*

A study of several chalimus forms shows that except for a slackening of the rate of growth in size there is little difference between this stage and that of the adult male (vide Figs.). But in the female there is not only a shrinkage in size but also change of outline of cephalothorax and genital segments. Examination of the reproductive organs shows that the ova are formed only in the post chalimus i.e. the adult, stage. This attainment of the maturity of the gonads is correlated with the assumption of the distinctly feminine external features whereas in the male, it is probable that the gonads ripen earlier and there is therefore not much alteration in external form.

General Remarks and Conclusions

The findings of Segerstrale (1937) from the studies of the amphipod *Pontoporeia affinis* only confirm the conclusions

of Stever (1931) based on the copepod *Rhinocalanus cornutus*. The adult male is shorter than the foregoing state and does not follow Brook's law of Fowler. This is borne out by the present study of the Chalimus and adult stages of *Caligus polycanthi.* The adult male is not proportionately larger than the preceding Chalimus; the female is even shorter in length and narrower about the cephalothorax. The extremely

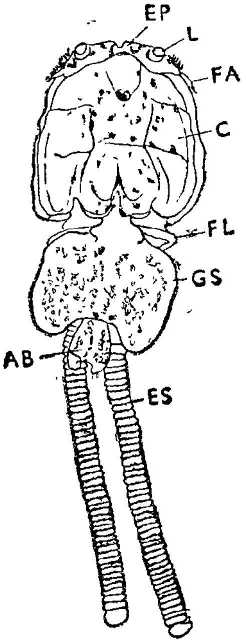


Fig. 3*a*. *Caligus polycanthi*
Mature female

A = Abdomen
C = Carapace
ES = Egg Sac
FA = First Antenna
FP = Frontal Plate
FL = Fourth Leg
GS = Genital Segment

small size of the male in forms like *Clavellisa* and *Bomolochus* can be explained by applying Sewell's analysis of size differences in the same sex. Sewell has found that during the growth of the copepod, the 'low' forms may attain the mature-adult stage while still small in size, whereas the 'high' forms are produced by the males passing through another stage of growth before becoming

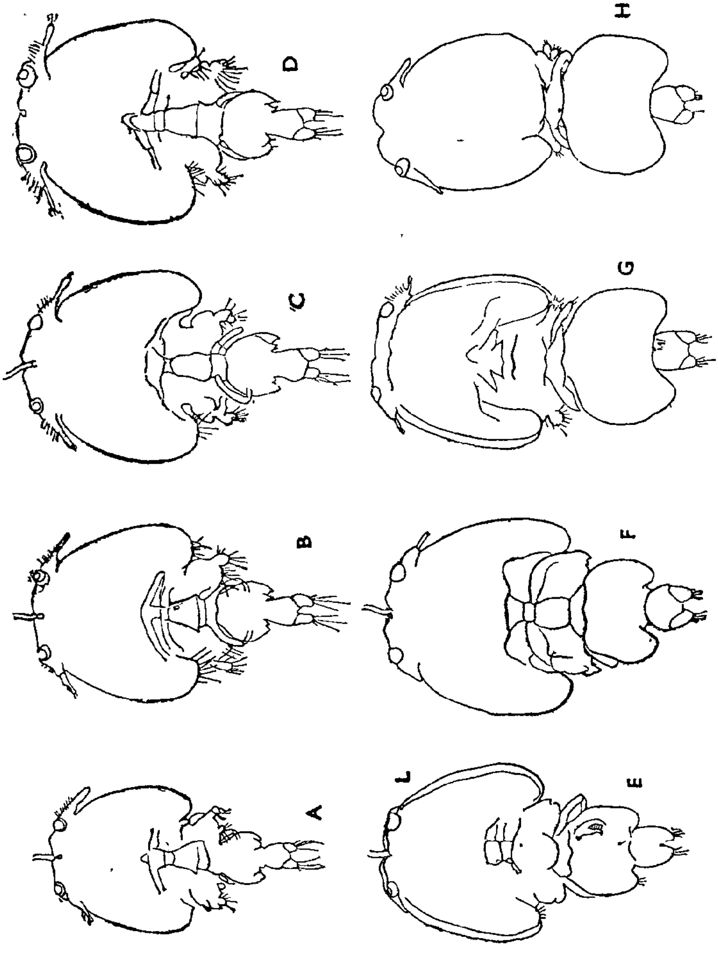


Fig. 3*b*. Chalimus and adult stages of male and female *Caligus polycanthi*
ABC = Chalimus stages (male)
D = Adult male
EFG = Chalimus stages (female)
H = Adult female

mature. The male of the parasitic species discussed above are probably equivalent to the 'low' forms of Sewell. The morphology of the male however is not likely to be less specialised and more indicative of the primitive features as is assumed by Wilson. The evidence from the study of the above forms is conflicting. The vestiges of the 6th appendages present in the female *Bomo-*

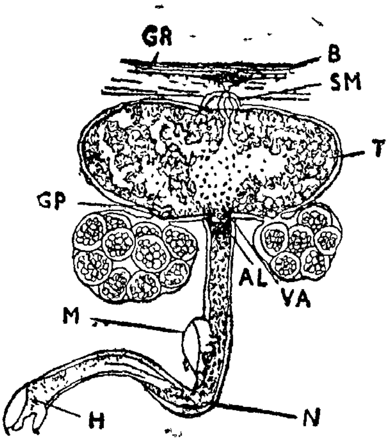


Fig. 4. Ventral view of entire parasite Clavellisa dussumieriae ×80.

AL = Anallaminae
B = Bulla
GP = Genital process
GR = Gill raker of fish
H = Head
M = Male
N = Neck
SIM = II Maxillae
T = Trunk
VA = Vestigial appendage

lochus multispinosa, are absent in the male which undoubtedly exhibits more primitive segmentation of the body. The male *Clavellisa* on the other hand has lost all traces of segmentation, remnants of the last thoracic limbs and anal laminae which are present in the female. Nevertheless that these degenerate features of the males are due to their attached mode of life rather than to their parasitic mode of feeding, is obvious since the males attached to the females, do not feed on the females.

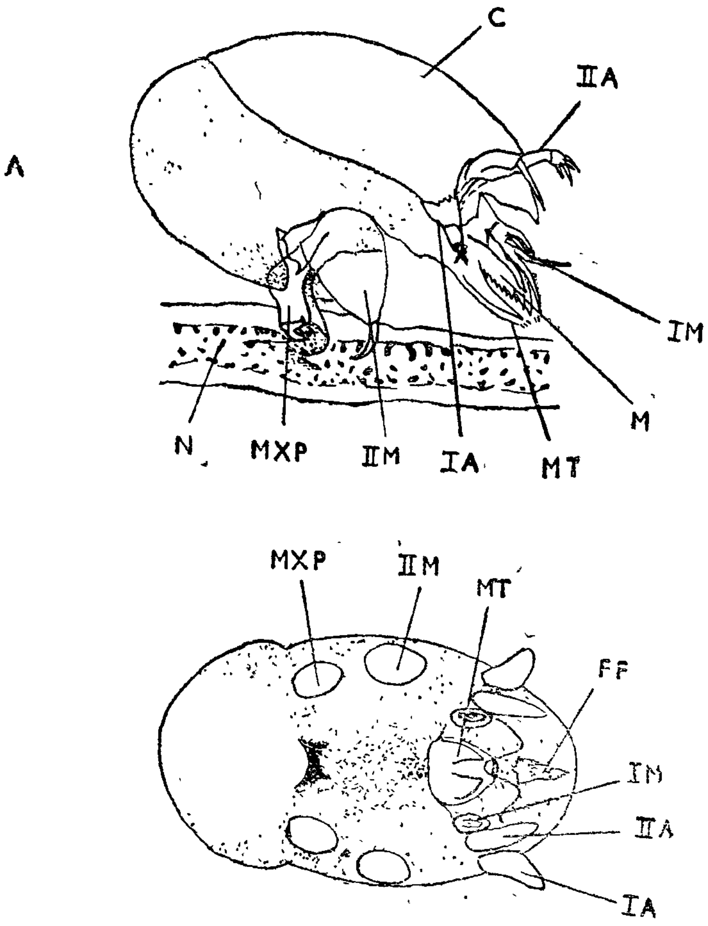


Fig. 5. A = Side view of male *Clavellisa dussumeriae*
B = Ventral view of male *Clavellisa Dussumeriae*

IA = I Antenna	FF = Frontal filament
IIA = II Antenna	M = Mandible
IM = I Maxilla	MT = Mouth Tube
IIM = II Maxilla	MXP = Maxillipede
C = Carapace	N = Neck of female

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A REVIEW OF PAST LOCUST INFESTATIONS IN SOUTH INDIA

BY

RAO BAHADUR Y. RAMACHANDRA RAO

In speaking of South India, one is often apt to consider it to be identical with the province of Madras. A look at a map of India would, however, show clearly that the Madras Presidency is mainly made up of the comparatively narrow strip of coastal country extending from the 8th parallel in the south to the 20th parallel in Ganjam (now included in Orissa), whereas South India should be held to include not only the greater part of Madras, but also the states of Mysore, Cochin and Travancore, as well as parts of the Hyderabad State and the southern districts of the Bombay Presidency. For our purposes, we may consider South India to be the country lying south of the 17th degree North Latitude, i.e., south of a line drawn roughly from Ratnagiri to Cocanada, passing through Bijapur and Hyderabad (Deccan).

LOCUST INVASIONS IN SOUTH INDIA

In view of the circumstance that the invasions of the Desert Locust are most frequently reported from North India, and that the outbreaks of the Bombay Locust affect mostly the central parts of India and the Deccan, it is generally taken as granted that the southern parts of India are immune from locust trouble. Although this is a fact, on the whole, for most years, a detailed study of the available records for the past eighty years and more has shown that locust visitations are by no means very uncommon. The Desert Locust swarms are known to have invaded South India on eight different occasions since 1861; incursions of the Bombay Locust are known to have reached parts of Madras Deccan, the northern districts of Mysore and the southern parts of Hyderabad State, whenever this species happened to be in a state of eruption in the Bombay area; and there was, moreover, a very serious development of the Migratory Locust in South India during the years 1877 – 1878.

I. *Invasions of the Desert Locust*: In the case of the Desert Locust, flights of its swarms are likely to reach South India at two different seasons: 1. One of these is during June-July, when South India may receive an extension of the summer flights of locusts of the spring brood. Usually flights of locusts bred in spring in Arabia, Iran and Baluchistan enter India in May-June, and may reach, often in conjunction with the Punjab-bred brood, the areas of Central Provinces, Bihar and Bengal under

the influence of the seasonal westerly winds. In some years, however, under the spell of a storm forming in the Bay of Bengal, northerly winds may develop in the areas of Central India, Berar and Central Provinces in May-June and carry the swarms southwards into the Hyderabad State and thence into the northern districts of Madras. Such an occurrence had apparently taken place in June, during the years 1891, 1893 and 1901 and the swarms would appear to have reached the Bellary and Kurnool districts (and also Guntur and Nellore in 1901), as well as Vizagapatam and Ganjam further north. 2. The second season is during the autumn months of September, October and November. At this time, swarms bred in the monsoon period in Rajputana and the Punjab are usually conveyed east and south-east by the prevailing westerly winds into the Central Provinces, in September-October. At this period also, there is a likelihood of the development of northerly or north-easterly winds under the influence of depressions forming on the Orissa-Circars coast, as a result of which swarms present in the area of Central Provinces and Berar would be carried southwards into Hyderabad and thence into Madras, or south-westwards into the Bombay Presidency. This is known to have occurred during the years 1878, 1889, 1890, 1930 and 1941. *During 1878*, swarms reached Bellary, Anantapur, Kurnool, Guntur, Kistna and Nellore in October. In November and December, some more swarms entered Guntur and Kistna districts from the Nizam's Territory, and it is on record that heaps of dead locusts were found in the fields, which had evidently perished of cold in the wake of a severe cyclone that struck the Kistna coast during December. *In 1889*, swarms would appear to have reached Bellary, Kistna, Godavari and Vizagapatam districts by November, some of which are apparently active in Kistna even in January, 1890. *In the autumn of 1890*, there was a great influx of locust flights from the Central Provinces that spread southwards across Ahmednagar and Poona into Bharwar, and thence into north Mysore, and into the districts of Bellary, Anantapur, Cuddapah, North Arcot, Nellore and Chingleput. The southernmost point reached was Trivellore (13°N. Lat.) about 30 miles west of Madras City. *After 1890*, there were no autumn visitations in the south till the 29th September, 1930, when swarms reached as far as Warangal in Hyderabad State. Recently, a few swarms would seem to have appeared in October, 1941 in the Belgaum district of Bombay and in the Bellary-Kurnool area in Madras.

II. *Infestations of the Bombay Locust*: If we analyse the known facts about the activities of the swarms of the Bombay Locust during years of its outbreaks, it is evident that they will fall under the following four heads:—1. Autumn flights from north to south from north Bombay and Central Provinces towards the southern parts of Bombay; 2. a period of comparative inactivity of swarms during the winter months in the forest areas

of the Western Ghats; 3. a resumption of swarm activity from April onwards, the flights generally moving towards the north or north-east in the direction of the northern parts of Bombay and into the Central Provinces under the influence of the South-westerly winds; and, 4. breeding in the northern parts of Bombay and in Central Provinces and Berar with the break of the monsoon.

So far as South India is concerned, swarms of the Bombay Locust appear there only during the months of May and June. In the Madras Presidency, they are observable mostly in the northern parts of Madras-Deccan, being seldom seen south of the Cuddapah district. As these areas receive little rainfall in June-July, there is no possibility of their breeding here. By the end of June, these swarms are no more to be seen in these areas. Possibly they pass on flying eastwards into the hill areas of the south-eastern parts of the Central Provinces, but there are no positive observations on record in respect of this matter.

The following are the actual available data in regard to their appearance in the past in South India. According to Buchanan (1807), reddish locust swarms were observed by him near Mandya in Mysore State in May, 1800. In May, 1865, swarms would appear to have passed over Nandigama in Kistna district from the direction of the Hyderabad State.

During May-June, 1880, locusts were reported from Hassan district (Hassan and Belur) and also from Shimoga district (Channagiri) in Mysore State, and from Bellary and Anantapur districts. In May, 1881, again, swarms would appear to have visited Bellary and Kurnool districts. In May, 1882, locusts were reported form Chittaldrug district in Mysore, and from several places in the Bellary and Kurnool districts, and in May, 1883 from Pattikonda in Kurnool district and from Anantapur. During May-June, 1884, numerous swarms were reported to have appeared in Kurnool, Bellary and Kistna districts coming south from the direction of the Nizam's territory, and later on in Tumkur, and Kolar districts in Mysore State, and in Anantapur and Cuddapah districts in Madras territory. All these data evidently pertain to the 1879–1884 outbreak cycle of the Bombay Locust in the Bombay area.

After 1884, there are no records for South India till May, 1902, when locust flights were sighted in the Bellary district. Similar flights were reported in successive years—always during the months of May and June—viz: 1903, 1904, 1905, 1906 and 1907, from parts of one or other of the following districts:—Bellary, Kurnool, Cuddapah and Anantapur. All these flights are referable to the infestation cycle of 1898–1908 of this locust in the Bombay Presidency. Since 1907, however, there have been no reports of flights in the Madras Presidency, which appears to be in consonance with the absence of outbreaks in the Bombay area since 1908,

A study of the above data in correlation with the items of information available for the areas of Bombay and Central Provinces would appear to indicate that the degree of incidence of the flights of this locust in South India is dependent partly on the relative abundance of the over-wintering swarms in the southern parts of the forest areas of the Western Ghats, and partly on the development of northerly winds during May-June deflecting the flights southwards from their normal north-easterly trends. On the whole, however, the flights of the Bombay Locust do not cause much damage in the Madras-Deccan areas, as at the time of their appearance in May-June, there are few crops to be seen there.

III. *Infestations of the Migratory Locust*: Though the solitary phase form of this locust is found all over India, the gregarious form would appear to be assumed but seldom under Indian conditions. So far as known, there are only two authentic records of swarming of this species in India.

Locust infestation in Gujarat in 1937: In September-October, 1937, damage to crops by locusts was reported from the Sirohi State in south Rajputana, and from the Mehsana, Palanpur, Idar and Kaira areas of north Gujarat, and an examination of the infested areas showed that the insects concerned were the *gregaria phase* hoppers of the Migratory Locust (*Locusta mirgatoria*, L.). As no concentrations of this locust had been noticeable in these areas prior to September, it was evident that the infestation had not been due to local developments. Fortunately, most of the desert areas of North-western India had been under close observation by the staff of the Locust Research Scheme of the Imperial Council of Agricultural Research since 1933, and the information collected by them in regard to the occurrence of heavy breeding of the solitary phase individuals of this species, *firstly* in April-May, 1937 in the hill-valleys of Baluchistan, and *later on* in July-August in the desert areas of east Bikaner and western Jaipur, has served to give valuable clues in regard to the probable origin of the September-October infestation of the Sirohi-Gujarat areas (Rao & Bhatia, 1939). A study of the meteorological factors has indicated that the solitary phase individuals bred in the Bolan and Kachhi areas of Baluchistan had migrated eastwards along with the prevailing south-west winds into the Bikaner-Jaipur areas by June, and that breeding had followed in these areas as the result of good and early monsoon rainfall. Consequently, a fairly dense population of the Migratory Locust was observed in these areas by the end of August, but with the appearance of a strong depression from the east during the first week of September, this was found to have disappeared from these areas, and it is surmised that a migration of individual locusts had occurred and that the cyclonic winds accompanying the depression had carried them into the Sirohi-

Gujarat areas in September, where concentrated oviposition had apparently followed and brought about the outbreak.

Locust Outbreak of 1878 in South India: Cotes (1891) has recorded the occurrence of a widespread locust infestation in South India during the year 1878, and a perusal of the original reports of this invasion preserved as printed Proceedings of the Board of Revenue, Madras, in the Madras Record Office was made with the kind permission of the Government of Madras (Development Department). Data on locust movements included in the Season and Crop Reports of the Gazette of India and of the official Gazettes of Madras, Mysore and Bombay were likewise studied, as a result of which fairly full and detailed information on the progress and decline of the outbreak of 1878 has been obtained. A preliminary account of the results of these studies was included in a recent publication (Rao, 1943), and a short resumè of the main events of this invasion is proposed to be given here.

A short account of the infestation of 1878: The available data on the outbreak cover a period of about a year—December, 1877 to December, 1878, and may be considered under the following sub-heads: 1. Winter breeding 1877–78, 2. Spring flights and breeding, 1878, and 3. Summer flights and breeding, 1878.

I. *Winter*—1877–78: The earliest information on record is in regard to heavy hopper infestation on Cholam and Cumbu crops in the Perambalur and Trichinopoly taluks of the Trichinopoly district in December, 1877. It is, however, probable that a report of damage by hoppers and locusts to Cholam in the Cuddapah district in November, 1877 is also referable to this species. In January-February, 1878 hopper attack on millet crops was reported from the Kamuty, Rajasingamangalam and Pallimadam taluks of Madura district, and from Ottapidaram and Sattur taluks of Tinnevelly district. At Tattapparai in Tinnevelly district, it was stated 'an army of red-brown creatures were seen marching slowly along eating every leaf of the *cumbu* crop' and at Paralachi in Madura district hoppers would appear to have been present in such large numbers that they could hardly be kept out of the houses. Apparently the hoppers became adults by February, when large flights of locusts began to be noticed. During the autumn and winter months, the prevalent winds in Madras are of the North-east Monsoon type and the gradual advance of the infestation into the southern districts had evidently been brought about by the agency of the north-east winds.

II. *Spring—1878*: Winged locusts appearing during January, February and March would seem to have formed into flights, which generally moved north or north-east, as the general wind direction at this part of the year is mostly southerly or south-easterly. Swarms originating in Tinnevelly passed into Madura, and from thence into Coimbatore and Malabar. During March and April, numerous flights were observed passing over the

Nilgiris and other hill-ranges into the western districts of Mysore and into Coorg. By April, swarms flying north from Chittaldrug and Shimoga districts entered the Bellary, Dharwar and Belgaum districts. Following good rainfall during April and May, in Coimbatore, Madura and Tinnevelly districts, in many parts of the Mysore State and in the Bellary district, egg-laying occurred in many places in these areas, and subsequently hoppers were reported damaging the crops. These assumed wings by the end of May and during June.

III. *Summer—1878*: From the middle of May, strong south-westerly winds commence blowing in these areas, and the young swarms of the spring brood, which began to assume wings by the end of May, were observed flying in a north-easterly direction in general. Consequently, the general movements of swarms was from Malabar, Coimbatore and Madura districts towards the east coast districts of Madras, viz: Trichinopoly, Tanjore, Salem, North Arcot, South Arcot, and Chingleput, and from Mysore areas into Chittoor, Cuddapah and Nellore, during the months of July and August. As the central and eastern districts of Madras normally receive only light rainfall during the South-west Monsoon period, there was very little breeding except in parts of Salem and Chittoor districts, and consequently it was observed that flights were gradually diminishing in size as well as in numbers. During September, 1878 comparatively few flights were observed, and during October, November and December, the few swarms that were reported were from the east coast districts of Chingleput, South Arcot, North Arcot and Tanjore. Unlike that of 1877, the north-east monsoon of 1878 proved defective, so that there was very little breeding. The infestation of 1878 thus came to an end.

The probable origin of the Outbreak: In the absence of a full knowledge of the habits and ecology of the solitary phase of this locust and its breeding areas in South India, it is difficult to make any surmise as to how the outbreak had originated. As the infestation had followed a period of drought and famine, there is little doubt that its origin should be sought in the abnormal conditions arising out of a prolonged drought in 1876–77 that was followed by an unusual burst of rainfall during the months of September, October and November, 1877. It is surmised that, during the period of drought, the breeding of migrating insects like locusts had been concentrated in the few places where local rainfall had occurred. As, usually, hill areas receive more rainfall than the plains, it is presumed that small concentrations of the Migratory Locust had formed on the grass areas of some of these hill areas. According to Uvarov (1936), the outbreaks of the Oriental Migratory Locust (*Locusta migratoria manilansis* Mey) in Borneo and the Philippines are associated with the grass areas that form on the hills as the result of shifting cultivation, it is not improbable that the outbreak centres involved in the

origination of the great infestation if 1878 had developed under somewhat similar conditions on some of the main hill ranges of Southern India. It is conceivable that incipient swarms produced on the hill flanks during 1876–77 had migrated into the eastern districts along with the South-west Monsoon current, and had bred in these areas during the heavy rains of September-October, 1877. Locusts produced here had possibly been swept down southwards in November-December, during the prevalence of the cyclonic storms of the North-east Monsoon, into the Trichinopoly, Madura and Tinnevelly areas, where dense egg-laying had apparently taken place, followed by the heavy hopper infestation noticed during December, 1877 and January-February, 1878.

Data for years after 1878: After 1878, there has been no similar serious infestation of the Migratory Locust in South India, but the following reports are recorded in some of the Proceedings of the Madras Board of Revenue in regard to the appearance of locusts:—1. A small swarm of locusts observed in April, 1881 in Madura district, 2. a swarm appearing in July, 1885 in Madura district, 3. another attacking sugarcane, *ragi* and paddy in August, 1885 in Trichinopoly district, 4. an instance of locust breeding in September, 1885 in South Arcot district, and 5. an attack of locusts on the gingelly crop in June, 1886 at Periakulam in Madura district. Though it is not possible to decide about the identity of the species of locusts concerned in all these cases from the available data, it should not be considered unlikely that some, at least, of these might have been instances of the activity of the Migratory Locust.

Though it is true that the Migratory Locust has not re-appeared in a serious form since 1878, it should be kept in mind that there is every likelihood of its recurrence if conditions should again be favourable for its development. If one takes into account the fact that this insect suddenly appeared in a pest condition in the Rajputana and Gujarat areas in 1937, it would be recognised that the possibility of its recurrence is not purely theoretical, and it would be but wisdom on our part to undertake a study of the seasonal distribution, the powers of migration, and the habits and ecology of the solitary phase of this species under the very varied conditions in which it is observed in the different parts of India. This would enable us to obtain a fuller knowledge of the conditions under which future outbreaks might develop, and to arrange to keep a close watch over the outbreak areas so as to check the infestation in its early stages.

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